Microbial load of frozen thawed Sahiwal semen extended in egg yolk, soya lecithin and liposome based extender


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ABSTRACT

Present investigation was carried out to study the bacterial load of different semen extender in frozen thawed semen samples of Sahiwal bulls maintained at Artificial Breeding Research Centre, ICAR-NDRI, Karnal, Haryana. Twenty four ejaculates from four bulls were collected during morning hours using sterilized artificial vagina and the ejaculates were evaluated. Semen samples showing more than 70 per cent progressive motility and having more than 800 million sperms per ml were used for further processing and freezing. The samples were split into six aliquots and extended in six different extenders [Conventional egg yolk (CEY), egg yolk of hen supplemented with omega enriched diet (group I), Egg yolk of hen supplemented with herbal feed (group II), 1% soya lecithin (SY1), commercially available Bioxcell (SY2) and Optixcell (LP)]. One ml of post thaw semen (four straws) was serially diluted (ten-fold) with normal saline, loaded in sterilized nutrient agar plate and incubated at 37°C for 48 hours. Number of colonies were counted in duplicate and multiplied by dilution factor. The Standard plate counts (CFU/ml) of frozen thawed semen samples extended in different extenders were analyzed by one way analysis of variance. The results revealed that bacterial load of SY1, SY2 and LP was significantly (p<0.05) different from egg yolk based extenders. The highest microbial load of frozen thawed semen samples was recorded in group II (646.30±71.65) egg yolk extender and lowest in optixcell (91.67±6.72) extender. Microbial load in all the extenders are well within the standard limit (5000CFU/ml) of frozen thawed semen sample. Lower bacterial load in present finding may be due to maintenance of better HACCP protocol for hygiene during preparation, semen collection and semen processing.

Key words: Bacterial load, Egg yolk, Liposome, Soya-lecithin, Sahiwal bull semen.

INTRODUCTION

Good quality disease free semen production and its propagation is one of the major goals of semen laboratories in India and throughout the world (Meena et al., 2015). High microbial loads not only affect semen quality (Diemer, et al., 1996) but also impact fertility status of spermatozoa. Bacteria compete with spermatozoa for nutrients and oxygen necessary for growth and normal functioning. Semen acts as a vehicle for a wide range of pathogens (Vinodh et al., 2007). Microbes enter into semen through number of routes from diseased animals, preputial sheath, extenders, as well as faulty procedure followed during collection, processing and packaging of semen. Microorganisms affect the male reproductive function directly by reducing the ability of acrosome reaction (Morrell, 2006) and agglutination of motile sperm and indirectly, by formation of reactive oxygen species generated by inflammatory response to the infection. Regular microbial evaluation of semen samples is a prerequisite to ensure the quality of semen. The rising issue of bio-security and cross boundary disease transmission has unveiled new concerns over the use of egg yolk based extenders, as they have been often incriminated for facilitating the transmission of some diseases. Various pathogens such as E. coli, Staphylococcus, Streptococcus, Pseudomonas, Haemophilus, Salmonella, Avian influenza, Campylobacter, Listeria and Mycoplasma are transmitted through egg yolk (Thibier and Guerin, 2000). Besides the risk of disease transmission, egg yolk globules interfere in the microscopic examination of the spermatozoa (Bousseau et al., 1998). Further, composition of egg yolk also varies upon their source, which prevents implementation of strict quality control of the extender. On the other side egg yolk inflicts damages to the chromatin integrity (Stradaioni et al., 2007). All these lead to an extensive search for an alternate semen extender, which is free from the risk of introducing exotic diseases. Now-a-days soya lecithin and liposome based extenders are commercially available but till date no proper validation is available regarding the microbial load status of soya lecithin and liposome based extenders. As per the World Organization for Animal Health

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MATERIALS AND METHODS

Experimental animals: Present investigation was conducted on Sahiwal bulls maintained at Artificial Breeding Research Centre, ICAR- NDRI, Karnal, India. All the bulls were maintained under standard management and nutritional conditions. A total of twenty four ejaculates (two ejaculates per week from each bull) were collected from four bulls during morning hours using artificial vagina. Immediately after collection, each ejaculate was placed in a water bath at 30-32°C throughout the evaluation. Each ejaculate was split into six parts and extended with different semen extenders for cryopreservation such as conventional egg yolk, egg yolk of hen supplemented with omega enriched diet (group I), egg yolk of hen supplemented with herbal medicines (group II), 1% soya lecithin, commercially available Bioxcell and Optixcell extender.

All semen samples were evaluated for quality traits for further cryopreservation at 196°C in liquid nitrogen. One ml of frozen thawed semen (approximately 4 straws) was diluted with 9 ml of normal saline solution or phosphate buffer using fresh autoclave tip for each successive dilution. Ten fold serial dilution of the semen sample (1:10, 1:100, 1:1000 and 1:10000) was made in sterile nutrient broth inside the laminar flow. Inoculums of 1ml from each dilution was diluted and then incubated at 37°C for 48 hours. C colonies per plate was recorded and counted with the help of colony counter. Colony forming unit (CFU) per ml of the sample were estimated by multiplying the dilution factor with the mean number of colonies in media plates (Bacterial load/ml=Bacterial colonies counted on plate ÷ dilution factor).

Statistical analysis: Data on bacterial load in different semen extenders were analyzed using SPSS statistical software package (Version 16.0). One-way analysis of variance was used to test the significance of extenders on the studied parameters. The pair wise difference of means between groups was compared by post hoc Tukey test and P-value less than 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

The least squares means of standard plate counts (CFU/ml) of frozen thawed semen samples extended in different extenders are presented in Table 1. Analysis revealed that, there was significant (p<0.05) difference between Soya lecithin, Bioxcell and Optixcell extenders and non-significant difference between group I and group II egg yolk extenders in comparison to conventional egg yolk extender. The highest microbial load of frozen thawed semen samples was recorded in group II egg yolk based extender, followed by conventional, group I egg yolk extender, Soya lecithin, Bioxcell extenders and lowest in optixcell extender (Table 1). The levels of microbial load in all the semen extenders are well within the standard limit (5000 CFU/ml) of frozen thawed semen samples. Present findings were in accordance with the earlier reports of Meena et al. (2010), who reported that bacterial load were significantly lower in Bioziphos as compared to conventional egg yolk extender in both pre-freeze and post-thaw stage in Murrah bulls. Similarly Bousseau et al. (1998) reported that two extenders containing animal products (Triladyl) and egg yolk plus milk-based extender (Laciphos) showed moderate (10 to 60 CFU/ml) contamination with bacteria or mycoplasma while no contamination in Bioziphos® lecithin containing diluents. In contrary to present findings, Sannat et al. (2015) reported higher bacterial load in frozen semen of cattle and buffalo bulls in conventional egg yolk based extender as compared to present values of microbial load in conventional egg yolk based extender in HF cross (1.1×10^3), Sahiwal (9.61×10^3), Gir (1.09×10^3), Red Sindhi (6.56×10^3), Jersey (1.92×10^3) and Tharparkar (6.11×10^3) bull. In comparison to present findings higher values of bacterial load was reported by Wierzbowski et al. (1984) in crossbred cattle bull (1.1×10^3 CFU/ml) and Patel et al. (2011) in different crossbred CB-576 (1.26×10^3 CFU/ml), CB-581 (3.49×10^3 CFU/ml), CB-585B (5.9×10^3 CFU/ml), CB-594 (1.1×10^3 CFU/ml) and pure Holstein Friesian (HF-439) bull (5.38×10^3 CFU/ml). This may be due to management problem in maintenance of hygiene starting from sterilization of materials used in semen collection and processing as microbial load in all the breeds showed very high values. Very high bacterial load as compared to present finding was reported by Rathnamma et al. (1997) in frozen semen (0.81 to 39 × 10^3 CFU/ml) of cow bull and Ronald and Prabhakar, (2001) in frozen semen (18.3×10^3 CFU/ml) . Rao et al. (2002) also reported that bacterial growth was significantly arrested in semen extended with biociphos plus as compared to Tris egg yolk based extender in Murrah and Jersey bulls. Higher bacterial load in egg yolk (conventional, group I and herbal group II) based extenders in comparison to soya lecithin and liposome extender may be due to presence of various organisms in

Table 1: Mean (±SE) values of Standard plate count (CFU/ml) of frozen thawed Sahiwal semen extended in different semen extenders

<table>
<thead>
<tr>
<th>Extenders</th>
<th>Standard plate count (CFU/ml)</th>
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<tbody>
<tr>
<td>Conventional egg yolk</td>
<td>562.90±72.57</td>
</tr>
<tr>
<td>Group I egg yolk</td>
<td>495.80±58.86</td>
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<tr>
<td>Group II egg yolk</td>
<td>646.30±71.65</td>
</tr>
<tr>
<td>Soya lecithin</td>
<td>356.70±37.12</td>
</tr>
<tr>
<td>Bioxcell</td>
<td>128.80±12.98</td>
</tr>
<tr>
<td>Optixcell</td>
<td>91.67±6.72</td>
</tr>
</tbody>
</table>

Means bearing different superscripts in a column differ significantly (p<0.05)
egg yolk (Thibier and Guerin, 2000). The fertilizing capacity of spermatozoa is negatively affected by the risk of microbial contamination associated with egg yolk (Boussau et al., 1998; Aires et al., 2003). Bacterial contamination is a major problem of egg yolk for extending of semen. Soya-lecithin is a non-animal origin and contamination free medium for the dissemination of semen (Ansari et al., 2013). Soya lecithin-based extender is comparable for bovine and rams semen cryopreservation in comparison to egg yolk-based extender (Aires et al., 2003). El-Keraby et al. (2010) reported that whole soybean milk replacing conventional egg yolk extender by increasing sperm motility and decreased bacterial count in post thawed bovine semen. Liposome is a chemically defined extender in terms of composition and structure and can be optimized by modulating their phospholipids composition or their size. Therefore, liposomes would be a better alternative to optimize the composition of semen extender and lower the risk of bacterial contamination. Washing of prepuce with KMnO$_4$ solution reduced microbial load and sperm abnormalities due to broad spectrum effect (Meena et al., 2015). Lower bacterial load in present findings in frozen semen samples may be due to preputial washing with antiseptic solution before collection as the preputial cavity is main source of semen contamination, use of open-ended sterilized artificial vagina, the use of semen extenders containing antibiotics, maintenance of better HACCP protocol for hygiene during preparation, semen collection and semen processing.

It can be concluded that bioxcell and optixcell were found to be superior as they had lower bacterial load in comparison to egg yolk based extenders. The higher microbial load of frozen thawed semen samples in egg yolk based extenders as compared to other extenders may be due to management conditions of the farm, but, it is well within the standard norms of microbial load. Therefore, optixcell and bioxcell extender may be used as an alternative to reduce the risk of transmitting various diseases of animal origin and adverse effect on sperm qualitative traits.

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REFERENCES


